

Claims

1. A method for the determination of apoptotic and/or necrotic conditions of living test cells comprising monitoring changes in the signal of a marker protein in said cells.
2. The method according to claim 1, wherein the change in the signal is monitored in the presence of a non-, pro-, or anti-apoptotically or necrotically active compound and/or a physical stimulus.
3. The method according to claim 1 or 2, wherein the marker protein is produced in the test cells after transfection of said cells with a DNA coding for and expressing the marker protein.
4. The method according to claim 3 wherein the test cells are stably transfected.
5. The method according to anyone of claims 1 to 4, wherein the marker protein is the green fluorescence protein (GFP) or a fluorescent mutant thereof.
6. The method according to claim 5, wherein the changes in the signal of GFP or the fluorescent mutant thereof is monitored by means of a flow cytometer or a platereader measuring the fluorescence intensity.
7. A method according to anyone of claims 1 to 6, wherein two groups of test cells are used, each of a defined number of cells, which were transfected with a DNA vector coding for a fluorescent marker protein, such as the GFP or a fluorescent mutant thereof, incubating one group together with the test compound in a culture medium, stimulating the cells of both groups with an excitation beam, determining the fluorescing intensities of the cells of each group by means of a flow cytometer, and comparing the changes in the fluorescing intensity of the cells of the two groups.
8. A method according to anyone of claims 5 to 7, wherein the GFP is introduced into the cells by the

DNA vector pBluescriptIIKS(+)+EF-1 α +EGFP or pEGFP-N1+MoLV-LTR.

9. A vector comprising a gene coding for a marker protein which is operably linked to one or more strong promoters, such as the hEF1- α promoter, the MoLV-LTR promoter or a combination of the CMV and the MoLV-LTR promoter.

10. The vector according to claim 9 wherein the gene codes for the GFP or a fluorescent mutant thereof.

11. The vector according to claim 10 wherein the gene codes for the marker protein GFPmut1.

12. The vector according to anyone of claims 9 to 11 which is pBluescriptIIKS(+)+EF-1 α +EGFP or pEGFP-N1-MoLV-LTR.

13. A live cell transfected with a vector according to anyone of claims 9 to 12.

14. A living cell line transfected with a vector according to claim 12 which is A20GFP, PB3cGFP, JurkatGFP, or DMGFP.

15. A method to assay the non-, pro- or anti-apoptotic or necrotic activity of a test compound and/or of a physical stimulus in living test cells comprising monitoring the change in the signal of a marker protein in said cells.

16. The method according to claim 15, comprising transfecting a group of said cells with a vector coding for and expressing a marker protein, treating the transfected cells in a suitable culture medium with the test compound, monitoring the changes in the signal of the expressed marker protein in said group of cells and comparing the results with the results observed with a parallel group of the same test cells which was not treated with the test compound.

17. The method according to claim 15 or 16, wherein the test compound comprises a multiplicity of

compounds, e.g. as obtained from combinatorical chemistry methods.

18. The method according to anyone of claims 15 to 17, wherein the cells are normal cells, infected cells or cancer cells.

19. The method according to anyone of claims 15 to 18, wherein the test cells are transfected with a vector according to anyone of claims 9 to 12.

20. The method according to claim 19, wherein the test cells are transfected with the vector pBluescriptIIKS(+)+EF-1 α +EGFP or pEGFP-N1+MoLV-LTR.

21. The method according to claim 19 or 20, wherein the test cells are cells from the transfected cell lines A20GFP, PB3cGFP, JurkatGFP, or DMGFP.

22. The method according to anyone of claims 15 to 21, wherein monitoring is performed with the aid of a flow cytometer, e.g. the FACScanTM.

23. The method according to anyone of claims 15 to 21, wherein monitoring is carried out by measuring the parameters FSC-Height, SSC-Height and the fluorescence of the marker protein and comparing the results after dot plot and/or histogram visualisation.

24. The method of anyone of claims 1 to 8 and 15 to 23 which is a drug screening method, a high throughput screening method and/or a large scale screening method.

25. Use of the methods of anyone of claims 1 to 8 and 15 to 23 for drug screening, high throughput screening and/or large scale screening.

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